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FILE 'REGISTRY' ENTERED AT 15:33:50 ON 24 JAN 2003

=> S TRANSGLUTAMINASE/CN
L1 3 TRANSGLUTAMINASE/CN

=> D 1-3

L1 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2003 ACS
RN 137741-97-0 REGISTRY
CN Blood-coagulation factor XIIIa, blood platelet-derived (9CI) (CA INDEX NAME)
OTHER NAMES:
CN ***Transglutaminase***
MF Unspecified
CI MAN
SR CA
LC STN Files: ADISNEWS, AGRICOLA, BIOSIS, CA, CAPLUS, CIN, PIRA, PROMT, TOXCENTER

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
11 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
11 REFERENCES IN FILE CAPLUS (1962 TO DATE)

L1 ANSWER 2 OF 3 REGISTRY COPYRIGHT 2003 ACS
RN 80146-85-6 REGISTRY
CN Glutamyltransferase, glutaminylpeptide .gamma.- (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Activa MP
CN Activa Supercurd
CN Activa TG
CN Activa TG-K
CN Activa TG-M
CN Activa TG-S
CN Activa TG-TI
CN Activa WM
CN Akuthiba TG-S
CN E.C. 2.3.2.13
CN Glutaminylpeptide .gamma.-glutamyltransferase
CN Polyamine transglutaminase
CN PPQ 6117
CN Tissue transglutaminase
CN ***Transglutaminase***
DR 300711-04-0
MF Unspecified
CI MAN
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CASREACT, CBNB, CEN, CHEMCATS, CHEMLIST, CIN, EMBASE, MSDS-OHS, PIRA, PROMT, RTECS*, TOXCENTER, USPAT2, USPATFULL
(*File contains numerically searchable property data)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
2633 REFERENCES IN FILE CA (1962 TO DATE)
26 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2638 REFERENCES IN FILE CAPLUS (1962 TO DATE)

L1 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2003 ACS
RN 9067-75-8 REGISTRY
CN Blood-coagulation factor XIIIa (9CI) (CA INDEX NAME)
OTHER NAMES:
CN Activated blood-coagulation factor XIII
CN Activated coagulation factor XIII
CN Blood-coagulation factor XIII, activated
CN Fibrin-crosslinking enzyme
CN Fibrinoligase
CN ***Transglutaminase***
MF Unspecified
CI MAN
LC STN Files: ADISNEWS, AGRICOLA, ANABSTR, BIOSIS, BIOTECHNO, CA, CAPLUS, CIN, EMBASE, PIRA, PROMT, TOXCENTER, USPATFULL

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*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
             489 REFERENCES IN FILE CA (1962 TO DATE)
               7 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
             490 REFERENCES IN FILE CAPLUS (1962 TO DATE)


FILE 'CAPLUS' ENTERED AT 15:34:42 ON 24 JAN 2003

=> S TRANSGLUTAMINASE;S L1;S L2,L3
          3595 TRANSGLUTAMINASE
           352 TRANSGLUTAMINASES
L2        3635 TRANSGLUTAMINASE
                 (TRANSGLUTAMINASE OR TRANSGLUTAMINASES)


L3        3064 L1


L4        3984 (L2 OR L3)

=> S RENATURE OR RE(W)NATURE;S REFOLD OR RE(W)FOLD;S ENZYME;S REACTIVATE OR RE(W)ACTIVATE;S ACID
           176 RENATURE
            60 RENATURES
           234 RENATURE
                 (RENATURE OR RENATURES)
        109320 RE
          8211 RES
        117231 RE
                 (RE OR RES)
        351708 NATURE
          1736 NATURES
        353186 NATURE
                 (NATURE OR NATURES)
             1 RE(W)NATURE
L5        235 RENATURE OR RE(W)NATURE


           474 REFOLD
           141 REFOLDS
           602 REFOLD
                 (REFOLD OR REFOLDS)
        109320 RE
          8211 RES
        117231 RE
                 (RE OR RES)
        378580 FOLD
          7787 FOLDS
        384698 FOLD
                 (FOLD OR FOLDS)
            14 RE(W)FOLD
L6        616 REFOLD OR RE(W)FOLD


        663419 ENZYME
        372868 ENZYMES
L7      830805 ENZYME
                 (ENZYME OR ENZYMES)


          1593 REACTIVATE
           417 REACTIVATES
          1979 REACTIVATE
                 (REACTIVATE OR REACTIVATES)
        109320 RE
          8211 RES
        117231 RE
                 (RE OR RES)
         49328 ACTIVATE
         29467 ACTIVATES
         74606 ACTIVATE
                 (ACTIVATE OR ACTIVATES)
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          34 RE(W)ACTIVATE
L8      2012 REACTIVATE OR RE(W)ACTIVATE


     3527472 ACID
     1348868 ACIDS
L9   3991026 ACID
               (ACID OR ACIDS)

=> S RENATURE? OR RE(W)NATURE?;S REFOLD? OR RE(W)FOLD?;S REACTIVATE? OR RE(W)ACTIVATE?
        1682 RENATURE?
      109320 RE
        8211 RES
      117231 RE
               (RE OR RES)
      353451 NATURE?
           7 RE(W)NATURE?
L10     1688 RENATURE? OR RE(W)NATURE?


        5431 REFOLD?
      109320 RE
        8211 RES
      117231 RE
               (RE OR RES)
      430349 FOLD?
          48 RE(W)FOLD?
L11     5467 REFOLD? OR RE(W)FOLD?


        9152 REACTIVATE?
      109320 RE
        8211 RES
      117231 RE
               (RE OR RES)
      451768 ACTIVATE?
         171 RE(W)ACTIVATE?
L12     9309 REACTIVATE? OR RE(W)ACTIVATE?

=> S L4 AND L10;S L4 AND L11;S L4 AND L12
L13        2 L4 AND L10


L14        7 L4 AND L11


L15        4 L4 AND L12

=> S L13,L14,L15
L16       12 (L13 OR L14 OR L15)

=> S L7 AND L10;S L7 AND L11;S L7 AND L12
L17      383 L7 AND L10


L18     1254 L7 AND L11


L19     2761 L7 AND L12

=> D L16 1-12 CBIB ABS;S (L17,L18,L19) AND L9

L16  ANSWER 1 OF 12  CAPLUS  COPYRIGHT 2003 ACS
2002:810334  In vitro   ***refolding***   process of urea-denatured microbial
       ***transglutaminase***   without pro-peptide sequence.  Yokoyama,
     Kei-ichi; Kunio, Ono; Ohtsuka, Tomoko; Nakamura, Nami; Seguro, Katsuya;
     Ejima, Daisuke (Central Research Laboratories, Ajinomoto Co. Inc.,
     Kawasaki, Kanagawa, 210-8681, Japan).  Protein Expression and
     Purification, 26(2), 329-335 (English) 2002.  CODEN: PEXPEJ.  ISSN:
     1046-5928.  Publisher: Elsevier Science.
AB   Efficient   ***refolding***   process of denatured mature microbial
```

transglutaminase (MTG) without pro-peptide sequence was studied in the model system using urea-denatured pure MTG. Recombinant MTG, produced and purified to homogeneity according to the protocol previously reported, was denatured with 8 M urea at neutral pH and rapidly dild. using various buffers. Rapid diln. with neutral pH buffers yielded low protein recovery. Redn. of protein concn. in the ***refolding*** soln. did not improve protein recovery. Rapid diln. with alk. buffers also yielded low protein recovery. However, diln. with mildly acidic buffers showed quant. protein recovery with partial enzymic activity, indicating that recovered protein was still arrested in the partially ***refolded*** state. Therefore, we further investigated the efficient ***refolding*** procedures of partially ***refolded*** MTG formed in the acidic buffers at low temp. (5 .degree.C). Although enzymic activity remained const. at pH 4, its hydrodynamic properties changed drastically during the 2 h after the diln. Titrn. of partially ***refolded*** MTG to pH 6 after 2 h of incubation at pH 4.0 improved the enzymic activity to a level comparable with that of the native enzyme. The same pH titrn. with incubation shorter than 2 h yielded less enzymic activity.

Refolding trials performed at room temp. led to aggregation, with almost half of the activity yield obtained at 5 .degree.C. We conclude that rapid diln. of urea denatured MTG under acidic pH at low temp. results in specific conformations that can then be converted to the native state by titrn. to physiol. pH.

L16 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2003 ACS

2002:623183 Document No. 137:335407 ***Transglutaminases*** and endocrine system. Dvorcakova, M.; Macejova, D.; Pallet, V.; Higueret, P.; Vasson, M.-P.; Rock, E.; Brtko, J. (Institute of Experimental Endocrinology, SAS, Bratislava, Slovakia). Endocrine Regulations, 36(1), 31-36 (English) 2002. CODEN: EREGE3. ISSN: 1210-0668. Publisher: Slovak Academic Press Ltd..

AB A review. ***Transglutaminases*** catalyze the posttranslation modification of proteins by catalyzing Ca2+ dependent acyl-transfer reaction resulting in the formation of new g-amide bonds between g-carboxamide groups of peptide-bound glutamine residues and various primary amines. Such glutamine residue serves as acyl-donor and the most common acyl-acceptors are e-amino groups of peptide-bound lysine residues or primary amino groups of some naturally occurring polyamines, like putrescine or spermidine. The active site of cysteine reacts first with the g-carboxamide group of glutamine residue to form the acyl-enzyme intermediate under the release of ammonia. In the second step, the complex reacts with a primary amine to form an isopeptide bond and liberate the ***reactivated*** enzyme. The presence of ***transglutaminases*** has been obsd. in various endocrine glands such as human pituitary which was investigated by immunohistochem. methods using specific antibodies. A significant increase in the expression and activity of tissues ***transglutaminase*** was obsd. during involution of thymus. In the genital tract of the male rat two different forms of the enzyme ***transglutaminase*** could be identified and characterized. the presence of p53 and tissues ***transglutaminase*** gene expressions in human normal and pathol. adrenal tissues. The Ca2+-responsive enzyme ***transglutaminase*** , which catalyzes the cross-bridging of proteins, was found in pancreatic islet cells.

L16 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2003 ACS

2001:455380 Document No. 135:104258 Polyethylene glycol enhanced ***refolding*** of the recombinant human tissue ***transglutaminase*** . Ambrus, Attila; Fesus, Laszlo (Department of Biochemistry and Molecular Biology, University of Debrecen, Debrecen, H-4012, Hung.). Preparative Biochemistry & Biotechnology, 31(1), 59-70 (English) 2001. CODEN: PBBIF4. ISSN: 1082-6068. Publisher: Marcel Dekker, Inc..

AB Tissue ***transglutaminase*** (I) forms crosslinks between Lys and Gln side-chains of polypeptide chains in a Ca2+-dependent reaction; its structural basis is still not clarified. Here, the authors demonstrate that the ***refolding*** of human recombinant I to its catalytically active form from inclusion bodies requires the presence of a helper material with higher mol. wt., but only in the initiation phase. Ca2+ and nucleotides are ascribed as effector mols. also in the early phase of structural reconstitution. Two optimal concns. of polyethylene glycol and a relatively long time scale for the evolution of the final structure were identified. The optimized ***refolding*** procedure is reported.

L16 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2003 ACS
2000:776898 Document No. 134:40326 Molecular chaperonic function of C-reactive protein induced by heating in HT-29 human colon carcinoma cells. Lee, Soo Young; Jung, Hyun-Jung; Kim, Hyun-Soo; Lee, Seung-Chul; Lee, Si-Back; Joe, Jae-Hoon; Kim, You-Mie (Department of Natural Sciences, Chemistry Section, College of Medicine, The Catholic University of Korea, Seoul, 137-701, S. Korea). Journal of Biochemistry and Molecular Biology, 33(5), 407-411 (English) 2000. CODEN: JBMBE5. ISSN: 1225-8687. Publisher: Springer-Verlag Singapore Pte. Ltd..

AB The effect of heat shock, or all-trans retinoic acid, on the expression of the C-reactive protein mRNA in the HT-29 human colon carcinoma cells, as well as the functional role of the C-reactive protein as a mol. chaperone, were studied. The expression level of the C-reactive protein mRNA in the HT-29 cells was increased time-dependently when exposed to heat-shock, and dose-dependently when treated with all-trans-Retinoic acid. The activities of ***transglutaminase*** C and K in the HT-29 cells were significantly increased when treated with all-trans retinoic acid. The C-reactive protein prevented thermal aggregation of the citrate synthase and stabilized the target enzyme, citrate synthase. The C-reactive protein promoted functional ***refolding*** of the urea-denatured citrate synthase up to 40-70%. These results suggest that the C-reactive protein, which is induced in human colon carcinoma cells, when heated or treated with all-trans-Retinoic acid has in a part functional activity of the mol. chaperone.

L16 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2003 ACS
2000:475778 Document No. 133:101387 Process for producing enzymatically active microbial ***transglutaminase*** via two step ***refolding***. Yokoyama, Keiichi; Ono, Kunio; Ejima, Daisuke (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2000040706 A1 20000713, 74 pp. DESIGNATED STATES: W: AU, BR, CA, CN, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1999-JP7250 19991224. PRIORITY: JP 1998-373131 19981228.

AB A method of producing enzymically active microbial ***transglutaminase*** (TGase) by subjecting ***transglutaminase*** in a denatured state to a ***refolding*** process involving at least the following steps (a) and (b): (a) the step of forming an intermediate structure, showing the enzymic activity in an aq. medium under acidic conditions, and (b) the step of forming a higher-order structure, showing the enzyme activity in an aq. medium at a neutral pH value. Cysteine thiol is preferably in a free reduced state, and TGase in soln. during the step (a). The process may addnl. include the treatment with agent that facilitate the higher-order structure formation, denaturing agent, and sepn. of inactive enzyme as aggregate. The intermediate structure should have 30 ~ 70% mol. ellipticity in near UV CD spectra. These are accomplished by adjusting the pH of enzyme contg. mildly acidic soln. to the neutral range.

L16 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2003 ACS
2000:466956 Document No. 133:248722 Overproduction of microbial ***transglutaminase*** in Escherichia coli, in vitro ***refolding***, and characterization of the ***refolded*** form. Yokoyama, Kei-Ichi; Nakamura, Nami; Seguro, Katsuya; Kubota, Kouji (Central Research Laboratories, Ajinomoto Co. Inc., Kanagawa, 210-0801, Japan). Bioscience, Biotechnology, and Biochemistry, 64(6), 1263-1270 (English) 2000. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.

AB The Streptoverticillium ***transglutaminase*** (MTG) gene, synthesized previously for yeast expression, was modified and resynthesized for overexpression in E. coli. A high-level expression plasmid, pUCTRPMTG-02(+), was constructed. Furthermore, to eliminate the N-terminal methionine, pUCTRPMTGD2 was constructed. Cultivation of E. coli transformed with pUCTRPMTG-02(+) or pUCTRPMTGD2 yielded a large amt. of MTG (200.apprx.300 mg/L) as insol. inclusion bodies. The N-terminal amino acid residue of the expressed protein was methionine or serine (the second amino acid residue of the mature MTG sequence), resp. Transformed E. coli cells were disrupted, and collected pellets of inclusion bodies were solubilized with 8 M urea. Rapid diln. treatment of solubilized MTG restored the enzymic activity. ***Refolded*** MTG, purified by ion-exchange chromatog., which had an N-terminal methionine or serine

residue, showed activity equiv. to that of native MTG. These results indicated that recombinant MTG could be produced efficiently in E. coli.

L16 ANSWER 7 OF 12 CAPLUS COPYRIGHT 2003 ACS
1997:348268 Document No. 127:76684 High-level expression of the chemically synthesized gene for microbial ***transglutaminase*** from Streptoverticillium in Escherichia coli. Kawai, Misako; Takehana, Shino; Takagi, Hiroshi (Central REs. Laboratoires, Ajinomoto Co., Inc., Kawasaki, 210, Japan). Bioscience, Biotechnology, and Biochemistry, 61(5), 830-835 (English) 1997. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.
AB We developed a novel approach for the high-level prodn. of a microbial ***transglutaminase*** (TGase) from Streptoverticillium in E. coli. The direct expression of the TGase gene in E. coli cells did not cause overprodn., probably due to the harmful influence of TGase activity, which introduces covalent crosslinks between proteins. Therefore, we fused the chem. synthesized TGase gene coding for the entire 331 amino acid residues at the amino terminus to a bacteriophage T7 gene 10 leader peptide (260 amino acids) using an inducible expression vector. The TGase gene was expressed as inclusion bodes in the E. coli cytoplasm. Restoring 15 amino acid residues upstream of the amino terminus of the mature TGase by a two-step deletion of the fusion sequence facilitated solubilization and subsequent proteolytic cleavage, thus releasing mature TGase. Although the mature form had less TGase activity than native TGase, because of the poor ***refolding*** rate, these results suggest that this system is suitable for the efficient prodn. of TGase.

L16 ANSWER 8 OF 12 CAPLUS COPYRIGHT 2003 ACS
1990:154313 Document No. 112:154313 Denaturation-renaturation of the fibrin-stabilizing factor XIIIa-chain isolated from human placenta. Properties of the native and reconstituted protein. Rinas, Ursula; Risse, Bernhard; Jaenicke, Rainer; Abel, Karl Josef; Zettlmeissl, Gerd (Inst. Biophys. Phys. Biochem., Univ. Regensburg, Regensburg, D-8400, Germany). Biological Chemistry Hoppe-Seyler, 371(1), 49-56 (English) 1990. CODEN: BCHSEI. ISSN: 0177-3593.
AB The denaturation-renaturation transition between the native and unfolded states of the dimeric blood coagulation factor XIIIa has been examd. by far-UV CD, fluorescence spectroscopy, activity measurements, sedimentation equil. anal., and size-exclusion HPLC. Guanidine hydrochloride and urea-dependent denaturation in the absence and in the presence of 5 mM dithioerythritol (DTE) or 5mM GSH exhibit biphasic transitions. The 1st stage represents a sharp transition characterized by a change in secondary structure without subunit dissocn. This step is accompanied by the irreversible loss of biol. activity. The 2nd transition reflects the dissocn. and complete unfolding of the protein to a random coil. After loss of biol. activity no reactivation can be accomplished under any of the following conditions: (1) denaturation and renaturation under reducing or non-reducing conditions; (2) variation of the protein concn. and temp.; (3) addn. of specific ligands (Ca2+, substrate); or (4) presence of stabilizing and/or destabilizing agents. Attempts to ***renature*** the protein under std. conditions (0.1M Tris/HCl pH 7.5-9.0, 5 mM DTE, 5 mM EDTA) lead to ***refolding*** intermediates which exhibit a strong tendency to aggregate. A sol. product of reconstitution can be obtained by ***refolding*** at low protein concn., low temp., and in the presence of small amts. of destabilizing agents such as arginine or urea in the renaturation buffer at pH 7.5-9. The spectroscopic and hydrodynamic characterization of the partially reconstituted (nonnative inactive) protein shows that partially reconstituted factor XIIIa exhibits the fluorescence properties and the dimeric structure of the native protein. On the other hand, the protein has a stronger tendency to stick to surfaces, indicating the exposure of hydrophobic parts that are buried in the authentic natural protein. A mechanism for the denaturation-renaturation process is proposed.

L16 ANSWER 9 OF 12 CAPLUS COPYRIGHT 2003 ACS
1989:151902 Document No. 110:151902 Effects of ***transglutaminase*** substrates and inhibitors on the motility of demembranated ***reactivated*** spermatozoa. De Lamirande, Eve; Gagnon, Claude (Urol. Res. Lab., R. Victoria Hosp., Montreal, QC, H3A 1A1, Can.). Gamete Research, 22(2), 179-92 (English) 1989. CODEN: GAMRDC. ISSN: 0148-7280.
AB The effects of ***transglutaminase*** (TGase) substrates, e.g.,

putrescine, dansylcadaverine, and spermine, and the TGase inhibitor cystamine were tested on the motility of demembranated mammalian spermatozoa. These products blocked within a few seconds the motility of demembranated ***reactivated*** spermatozoa at concns. ranging 0.25-5 mM. These minimal inhibitory concns. could be decreased 5-150-fold when TGase substrates and inhibitor were incubated with demembranated spermatozoa for 15 min prior to the addn. of Mg-ATP. The inhibition was reversed by higher concns. of Mg-ATP, but none of these TGase substrates or inhibitor could inhibit bull sperm dynein ATPase. TGase activities, as measured by the incorporation of [3H]putrescine into TCA-precipitable proteins, were present in both sperm Triton-sol. and -insol. fractions. On the other hand, amine acceptor protein substrates for the TGase-catalyzed reaction were present only in the insol. fraction. The Triton-sol. TGase was similar to the known tissue TGases; the Triton-insol. TGase activity was Ca independent. The same TGase substrates and inhibitor that blocked the motility of ***reactivated*** spermatozoa also blocked TGase activities. Linear relations were obsd. between the concns. of these substances required to block sperm motility and those to block TGase activities. These data suggest the involvement of a TGase activity in sperm motility.

L16 ANSWER 10 OF 12 CAPLUS COPYRIGHT 2003 ACS
1981:564569 Document No. 95:164569 ***Transglutaminase*** -catalyzed incorporation of putrescine into denatured cytochrome c. Preparation of a mono-substituted derivative reactive with cytochrome c oxidase. Butler, Stephen J.; Landon, Michael (Med. Sch., Univ. Nottingham, Nottingham, NG7 2UH, UK). Biochimica et Biophysica Acta, 670(2), 214-21 (English) 1981. CODEN: BBACAQ. ISSN: 0006-3002.
AB Guinea pig liver ***transglutaminase*** has been used to incorporate putrescine into horse heart cytochrome c. The native protein showed essentially no incorporation, whereas EtOH-denatured cytochrome c incorporated almost 1 mol putrescine/mol protein. No increase in this level of modification was obtained when maleylated cytochrome c and the tryptic peptides of cytochrome c were used as substrates. Anal. of the modified EtOH-denatured cytochrome c by tryptic cleavage and peptide isolation showed that glutamine-42 of the intact protein is the site of incorporation of radioactively labeled putrescine. EtOH-denatured cytochrome c that was specifically modified at glutamine-42 by incorporation of putrescine could be readily ***renatured*** . The ***renatured*** modified protein showed reactivity with cytochrome oxidase comparable to that of the original native protein.

L16 ANSWER 11 OF 12 CAPLUS COPYRIGHT 2003 ACS
1969:419041 Document No. 71:19041 Mechanism of the inactivation of guinea pig liver ***transglutaminase*** by 5,5'-dithiobis-(2-nitrobenzoic acid). Connellan, John M.; Folk, J. E. (Nat. Inst. of Dent. Res., Nat. Inst. of Health, Bethesda, MD, USA). Journal of Biological Chemistry, 244(12), 3173-81 (English) 1969. CODEN: JBCHA3. ISSN: 0021-9258.
AB Reaction of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) with ***transglutaminase*** in the absence of Ca2+ results in losses in the transferase and hydrolysis activities of the enzyme toward the substrate, benzyloxycarbonyl-L-glutaminylglycine (I). These activities are reduced 70-100% by reaction with 1-1.5 equivs. of DTNB. The Ca2+-dependent esterase activity of ***transglutaminase*** toward p-nitrophenyl acetate is not lost by this treatment. However, the activator const. of Ca2+ for this esterase activity, 1.7 .times. 10-3M at pH 7, is several-fold higher than that observed for the esterase activity of native enzyme. The esterase activity of DTNB-modified enzyme is not inhibited by I. This is in contrast to the effective inhibition afforded by this substrate toward the esterase activity of native ***transglutaminase*** . I protects the enzyme against inactivation by DTNB. This protection is observed only in the presence of Ca2+, which is essential for the binding of this substrate. Complete loss in transferase and hydrolysis activities is accompanied by the loss of 2 SH residues in the enzyme and the concomitant release of 2 equivs. of 5-thio-2-nitrobenzoic acid (or its thioquinone) for each equiv. of DTNB used for the modification. The loss in SH groups appears to be the result of formation of a single intramol. SS bridge in the enzyme. The DTNB-inactivated enzyme is readily ***reactivated*** by treatment with dithiothreitol. Peptide mapping studies show that a single SH group of ***transglutaminase*** previously identified as essential for all catalytic activities is not a

component of the SS bridge formed as a result of DTNB treatment. The changes that occur in ***transglutaminase*** upon treatment with DTNB in the absence of Ca2+ evidently result in a loss in binding properties for glutamine substrate. In contrast to the inactivation of ***transglutaminase*** by low levels of DTNB in the absence of Ca2+, inactivation of the enzyme at a high Ca2+ concn. (50mM) requires several-fold higher DTNB levels. Furthermore, DTNB treatment at high Ca2+ levels results in parallel losses in all 3 catalytic activities of the enzyme. This finding is in accord with earlier observations that a Ca2+-induced conformational change in ***transglutaminase*** affects the reactivity of the enzyme protein toward modifying agents.

L16 ANSWER 12 OF 12 CAPLUS COPYRIGHT 2003 ACS
1969:34607 Document No. 70:34607 A reversible, calcium-dependent, copper-catalyzed inactivation of guinea pig liver ***transglutaminase***. Boothe, R. L.; Folk, John E. (Nat. Inst. of Dent. Res., Nat. Inst. of Health, Bethesda, MD, USA). Journal of Biological Chemistry, 244(2), 399-405 (English) 1969. CODEN: JBCHA3. ISSN: 0021-9258.
AB Guinea pig liver ***transglutaminase*** is rapidly and progressively inactivated during incubation with Cu salts in the presence of Ca, a divalent cation essential for enzymatic activity. Substrate exerts some protection against this inactivation. Complete loss of ***transglutaminase*** activity is accompanied by a loss of .apprx.4 SH residues, probably by the formation of 2 intramol. S-S bridges. The Cu++-inactivated enzyme is readily ***reactivated*** by treatment with dithiothreitol or KCN. Peptide mapping expts. show that a SH group of ***transglutaminase*** previously identified as essential for catalytic activity is not a component of the S-S bonds formed as a result of Cu++ treatment. The dissocn. const. (K D) for Ca (7.1 .+-. 1.0 .times. 10-3 M) estd. from the rate of inactivation of ***transglutaminase*** by Cu++ as a function of Ca++ concn. is in close agreement with K D values for Ca detd. by other direct binding expts. A Ca-induced conformational change in the enzyme protein may be essential for Cu++-catalyzed formation of an inactive S-S form of ***transglutaminase*** .

L20 1544 ((L17 OR L18 OR L19)) AND L9

=> S (L17,L18,L19) (4A) L9
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED ') (4A) L9'
L21 1544 ((L17 OR L18 OR L19)) (4A) L9

=> S L7(4A)L10;S L7(4A)L11;S L7(4A)L12
L22 89 L7(4A)L10

L23 274 L7(4A)L11

L24 943 L7(4A)L12

=> S L22 (4A)L9;S L23(4A)L9;S L24(4A)L0
L25 0 L22 (4A)L9

L26 2 L23(4A)L9

2396 L0
L27 0 L24(4A)L0

=> S L24(4A)L9
L28 23 L24(4A)L9

=> S L26,L28
L29 25 (L26 OR L28)

=> S L29 NOT L16
L30 25 L29 NOT L16

=> D 1-25 CBIB ABS

L30 ANSWER 1 OF 25 CAPLUS COPYRIGHT 2003 ACS
1999:89927 Document No. 130:235193 Short-term regulation of carnitine palmitoyltransferase I in cultured rat hepatocytes: spontaneous inactivation and reactivation by fatty acids. Sleboda, Jowita; Risan, Kari Anne; Spydevold, Oystein; Bremer, Jon (Institute of Medical Biochemistry, University of Oslo, Oslo, 0317, Norway). Biochimica et Biophysica Acta, 1436(3), 541-549 (English) 1999. CODEN: BBACAQ. ISSN: 0006-3002. Publisher: Elsevier Science B.V..
AB Liver carnitine palmitoyltransferase I (CPT I), the rate-limiting enzyme of mitochondrial .beta.-oxidn., rapidly loses its activity when hepatocytes are put in culture. 3-Thia fatty ***acids*** ***reactivate*** the ***enzyme*** and can increase its activity 3-4-fold in 5-10 min. Normal fatty acids are also able to stimulate CPT I, but to a limited extent, compared to 3-thia fatty acid. This activation does not affect malonyl-CoA sensitivity. CPT I in hepatocytes from both fasted and fasted-carbohydrate refed rats is inactivated and reactivated to a similar extent. Free dodecylthioacetic acid (DTA) is at least as efficient as DTA-CoA as activator. CPT I activity in isolated mitochondria is not influenced by incubation with DTA, suggesting that the regulation of CPT I depends on an extramitochondrial component(s) in the cell. It is concluded that fatty acids activate pre-existing, inactive CPT I without involvement of gene transcription and independently of malonyl-CoA.

L30 ANSWER 2 OF 25 CAPLUS COPYRIGHT 2003 ACS
1998:290910 Document No. 129:25641 Transgenically produced glycinebetaine protects ribulose 1,5-bisphosphate carboxylase/oxygenase from inactivation in Synechococcus sp. PCC7942 under salt stress. Nomura, Mika; Hibino, Takashi; Takabe, Teruhiro; Sugiyama, Tatsuo; Yokota, Akiho; Miyake, Hiroshi; Takabe, Tetsuko (BioScience Center, Nagoya University, Nagoya, 464-01, Japan). Plant and Cell Physiology, 39(4), 425-432 (English) 1998. CODEN: PCPHA5. ISSN: 0032-0781. Publisher: Japanese Society of Plant Physiologists.
AB Synechococcus sp. PCC7942 cells transformed with Escherichia coli bet genes accumulated glycinebetaine (to about 80 mM) and acquired an increased tolerance to salt stress. Ribulose 1,5-bisphosphate carboxylase activity in the ext. from control cells was found to decrease more rapidly than that of either PSI or PSII under salt stress. We examd. levels of the transcript, the protein and the enzyme activity of ribulose 1,5-bisphosphate carboxylase to det. at which level the enzyme is affected at the early stages of salt stress. The levels of rbcL mRNA in both control and bet-contg. cells increased around two-fold under salt stress compared to those under non-stress. Bet-contg. cells showed slightly reduced levels of rbcL mRNA under both non-stress and salt stress compared to control cells. The protein levels of the enzyme in the control and bet-contg. cells were almost the same and were little changed by salt stress. On the other hand, salt stress drastically decreased the enzyme activity in control cells, but not as much in the bet-contg. cells. Interestingly the lowered enzyme activity in exts. from control cells grown under salt stress, as well as ***acid*** -denatured ***enzyme*** , was partially ***reactivated*** by glycinebetaine.

L30 ANSWER 3 OF 25 CAPLUS COPYRIGHT 2003 ACS
1998:264674 Document No. 129:64446 The effect of the intersubunit disulfide bond on the structural and functional properties of the small heat shock protein Hsp25. Zavialov, Anton; Benndorf, Rainer; Ehrnsperger, Monika; Zav'yalov, Vladimir; Dudich, Igor; Buchner, Johannes; Gaestel, Matthias (Institute of Immunological Engineering, Lyubuchany, 142380, Russia). International Journal of Biological Macromolecules, 22(3,4), 163-173 (English) 1998. CODEN: IJBMDR. ISSN: 0141-8130. Publisher: Elsevier Science Ltd..
AB The murine small heat shock protein Hsp25 carries a single cysteine residue in position 141 of its amino acid sequence. Interestingly, Hsp25 can exist within the cell as covalently bound dimer, which is linked by an intermol. disulfide bond between two monomers. Oxidative stress caused by treatment of the cells with diamide, arsenite, or hydrogen peroxide leads to an increase in Hsp25 dimerization, which can be blocked by simultaneous treatment with reducing agents. Recombinant Hsp25 was prepd. in an oxidized dimeric (oxHsp25) and reduced monomeric (redHsp25) form. The two

species were compared with regard to secondary structure, stability, oligomerization properties and their chaperone activity. It is demonstrated by CD measurements in the far UV region that there are no significant differences in the secondary structure and temp.- or pH-stability of oxHsp25 and redHsp25. However, according to CD measurements in the near UV region, an increase in the asymmetry of the microenvironment of arom. residues in oxHsp25 is obsd. Furthermore, an increase in stability of the hydrophobic environment of the tryptophan residues mainly located in the N-terminal domain of the protein against urea denaturation is detected in oxHsp25. Both reduced and oxidized Hsp25 form oligomeric complexes of similar size and stability against detergents, and both species prevent thermal aggregation of citrate synthase and assist significantly in oxaloacetic ***acid*** -induced ***refolding*** of the ***enzyme*** . Hence, the overall secondary structure, the degree of oligomerization and the chaperone activity of Hsp25 seem independent of the formation of the intermol. disulfide bond, and only the stability of the hydrophobic N-terminal part of the mol. is influenced by formation of this bond. The obtained data do not exclude the possible involvement of dimerization of this protein in other cellular functions, e.g. in intracellular sulfhydryl-buffering or in the protection of actin filaments from fragmentation upon oxidative stress.

L30 ANSWER 4 OF 25 CAPLUS COPYRIGHT 2003 ACS

1995:828277 Document No. 123:221586 Reversible inhibition of NADPH-cytochrome P450 reductase by .alpha.-lipoic acid. Slepneva, Irina A.; Sergeeva, Svetlana V.; Khramtsov, Valery V. (Inst. Chem. Kinetics Combustion, Novosibirsk, 630090, Russia). Biochemical and Biophysical Research Communications, 214(3), 1246-53 (English) 1995. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Academic.

AB NADPH-cytochrome-P 450 reductase both purified from rat hepatic microsomes and involved in microsomal fraction was inactivated by treatment with .alpha.-lipoic acid. Since .alpha.-lipoic acid contains disulfide bond, it reacts with SH-groups of the reductase via the reaction of thiol-disulfide exchange resulting in the loss of the enzyme reducing activity. NADP+ completely protected reductase from the inactivation. The modification of reductase was reversible: the modified ***enzyme*** was partially ***reactivated*** with dithiothreitol and dihydrolipoic ***acid*** in the case when cytochrome c was used as a substrate of reductase. In the case when inorg. substrate, K3Fe(CN)6, was used for assay the activity of modified reductase no reactivation was obsd. It was found that the order of the reaction of inactivation of membrane-bound microsomal reductase is equal to 1.2, which is in an agreement with pseudo-first order kinetics, and the second-order-rate const. of 26 M-1 min-1. The results have shown that well known therapeutic agent .alpha.-lipoic acid is an efficient inhibitor of both purified and microsomal reductase.

L30 ANSWER 5 OF 25 CAPLUS COPYRIGHT 2003 ACS

1984:2572 Document No. 100:2572 Bovine liver dihydropyrimidine amidohydrolase: purification, properties, and characterization as a zinc metalloenzyme. Brooks, Kathleen P.; Jones, Evan A.; Kim, Byung Dong; Sander, Eugene G. (Med. Cent., West Virginia Univ., Morgantown, WV, 26506, USA). Archives of Biochemistry and Biophysics, 226(2), 469-83 (English) 1983. CODEN: ABBIA4. ISSN: 0003-9861.

AB Beef liver dihydropyrimidine amidohydrolase (I) was purified to homogeneity by using both an electrophoretic and a hydrophobic chromatog. method. The enzyme is a tetramer with a mol. wt. of 226,000, a subunit mol. wt. of 56,500, and contains 4 mol of tightly bound Zn2+/mol of active enzyme. I appears to be a true Zn2+ metalloenzyme because there exists a direct proportionality between enrichment of Zn2+ and active enzyme during purifn., there is an almost quant. relation between the loss of both I activity and Zn2+ during 8-hydroxyquinoline-5-sulfonic acid treatment to form apoenzyme. Zn2+ and Co2+ ***reactivate*** dipicolinic ***acid*** -inhibited ***enzyme*** , and satg. concns. of a substrate, dihydrothymine, protect against 8-hydroxyquinoline-5-sulfonic acid inhibition. EDTA does not inhibit the enzyme; however, 8-hydroxyquinoline-5-sulfonic acid, o-phenanthroline, and 2,6-dipicolinic acid cause a time-dependent loss in activity which follows pseudo-1-order kinetics. Anal. of the resulting kinetic data for these 3 chelators indicates that the reaction pathway involves the formation of I-Zn2+-chelator ternary complex which then dissocs. to form apoenzyme and

a Zn2+-chelator complex. Like the other Zn2+ metalloenzymes, I is inhibited by a no. of substituted sulfonamides. In the case of p-nitrobenzensulfonamide, this inhibition is competitive in nature. By using the purified enzyme, kinetic consts. were detd. for a variety of dihydropimidines, unreidocarboxylic acids, and hydantoin substrates. Normal hyperbolic kinetics were obsd. for the hydrolysis of the cyclic compds., but the cyclization of the ureidoacids showed biphasic kinetics and different values of Km can be estd. at either high or low concns. of these substrates.

L30 ANSWER 6 OF 25 CAPLUS COPYRIGHT 2003 ACS
1982:419636 Document No. 97:19636 Characterization of 3-guanidinopropionate amidinohydrolase from Pseudomonas aeruginosa and a comparative study with 4-guanidinobutyrate amidinohydrolase from another Pseudomonas. Yorifuji, Takamitsu; Sugai, Ichiro; Matsumoto, Hideki; Tabuchi, Akira (Dep. Agric. Biol. Chem., Shinshu Univ., Nagano, 399-45, Japan). Agricultural and Biological Chemistry, 46(5), 1361-7 (English) 1982. CODEN: ABCHA6. ISSN: 0002-1369.
AB 3-Guanidinopropionate amidinohydrolase, a new enzyme (EC class 3.5.3), was purified 220-fold from P. aeruginosa PAO 1 grown on 3-guanidinopropionate. The enzyme was essentially homogeneous on polyacrylamide gel electrophoresis, with a mol. wt. of 195,000-215,000. The subunit mol. wt. was 36,000. The optimal pH was 9.0. The Km value for 3-guanidinopropionate was 45 mM. Incubation with EDTA in K phosphate buffer, pH 7.0, at 40.degree. resulted in almost complete inactivation, and the inactive enzyme was specifically reactivated by Mn2+. Taurocyamine (11%) and 4-guanidinobutyrate (3%) were hydrolyzed as fast as 3-guanidinopropionate at the relative rates indicated. The enzyme was inactivated by p-chloromercuribenzoic ***acid*** and the inactive ***enzyme*** was ***reactivated*** by incubation with 2-mercaptoethanol. Coelectrophoresis of the enzyme with 4-guanidinobutyrate amidinohydrolase purified from Pseudomonas species ATCC 14676 in polyacrylamide gels in the presence and absence of SDS demonstrated their close mobilities. 4-Aminobutyrate, propionate, and n-butyrate were common competitive inhibitors of these enzymes. The evolutionary relation between the 2 enzymes is discussed.

L30 ANSWER 7 OF 25 CAPLUS COPYRIGHT 2003 ACS
1980:507537 Document No. 93:107537 Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by dexamethasone not mediated by phosphorylation and dephosphorylation. Ramachandran, Chittoor K.; Gray, Susan L.; Melnykovych, George (Dep. Microbiol., Univ. Kansas Med. Cent., Kansas City, KS, 66103, USA). Archives of Biochemistry and Biophysics, 203(1), 117-22 (English) 1980. CODEN: ABBIA4. ISSN: 0003-9861.
GI

/ Structure 1 in file .gra /

AB The activity of 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.34) [9028-35-7] was increased several-fold in the microsomes isolated from HeLa S3G cells grown in the presence of 10-6M dexamethasone (I) [50-02-2]. EC1.1.1.34 activity was inactivated by ATP [56-65-5] + Mg2+. This inactivation was a function of time of incubation and ATP + Mg2+ concn. Nevertheless I-treated cells always showed higher activity. The inactivated ***enzyme*** was ***reactivated*** by incubation with potato ***acid*** phosphatase [9001-77-8]. Incubation of the microsomes with either purified alk. phosphatase [9001-78-9] from intestine or HeLa cell homogenates as a source of alk. phosphatase showed no effect on the activity of EC 1.1.1.34. ML236B, a competitive inhibitor of the enzyme, partially inactivated it. Both in the presence and in the absence of this compd., I-treated cells showed higher activity. However, the relative percentage inhibitions by ML236B at a fixed enzyme concn. remained the same for both control and I-treated cells. Apparently, the increase in EC 1.1.1.34 activity elicited by I is due to the increased level of the enzyme.

L30 ANSWER 8 OF 25 CAPLUS COPYRIGHT 2003 ACS
1978:611052 Document No. 89:211052 Characterization of the lipids involved in the (sodium-potassium) ion-activated and calcium ion-activated ATPases

in the human erythrocyte membrane by using highly purified phospholipases. Roelofsen, Ben (Lab. Biochem., Univ. Utrecht, Utrecht, Neth.). Proceedings of the FEBS Meeting, Volume Date 1977, 45(Membr. Proteins), 183-90 (English) 1978. CODEN: FEBPBY. ISSN: 0071-4402.

AB The Na+, K+-ATPase activity of delipidated red cell ghosts was restored by phosphatidylserine (PS) to >100% of the control value. Phosphatidic ***acid*** also ***reactivated*** the ***enzyme*** , although less effectively than PS. Addn. of a total phospholipid mixt. from human erythrocytes only caused a reactivation of .apprx.25% which is probably due to PS, since phosphatidylcholine (PC) and phosphatidylethanolamine (PE) alone cause no reactivation. Dicetylphosphate was also ineffective. Considerable activation of Ca2+, Mg2+-APase in delipidated ghosts was achieved by PC, PE, or PS, although the latter has the greatest effect. A mixt. of PC + PE + PS was as effective as PS alone. Lysolecithin was also able to bring about .apprx.50% reactivation. Free fatty ***acids*** not only failed to ***reactivate*** the ***enzyme*** but strongly impaired the reactivating capability of lysolecithin, esp. at higher lipid:protein ratios. From these results and previous studies with phospholipases, it is concluded that the Ca2+, Mg2+-ATPase of human erythrocyte membrane is dependent upon the total glycerophospholipid complement of the membrane and particularly upon that fraction forming part of the inner monolayer. The Na+, K+-ATPase has an abs. requirement for neg. charged diacylglycerophosphates but individual differences as to which neg. charged glycerophospholipid is involved may exist among various membrane species.

L30 ANSWER 9 OF 25 CAPLUS COPYRIGHT 2003 ACS

1976:505755 Document No. 85:105755 Creatine kinase activity in serum and uric acid solution. Warren, William A.; Madelian, Vergine (Div. Lab. Res., New York State Dep. Health, Albany, NY, USA). Clinica Chimica Acta, 70(2), 285-8 (English) 1976. CODEN: CCATAR. ISSN: 0009-8981.

AB Skeletal muscle creatine kinases from man and rabbit, activated to measured sp. activities by thiol agents, were inactivated in human serum and in uric acid soln., and the extent of their subsequent reactivation by dithiothreitol was detd. Both ***enzymes*** were completely ***reactivated*** from uric ***acid*** soln. but were only 60-70% reactivated from serum. No inactivation occurred within 14 days if serum samples contained dithiothreitol (0.01M) and were stored at -17.degree..

L30 ANSWER 10 OF 25 CAPLUS COPYRIGHT 2003 ACS

1974:117711 Document No. 80:117711 Fluorotyrosine alkaline phosphatase from Escherichia coli. Preparation, properties, and fluorine-19 nuclear magnetic resonance spectrum. Sykes, Brian D.; Weingarten, Harold I.; Schlesinger, Milton J. (Dep. Chem., Harvard Univ., Cambridge, MA, USA). Proceedings of the National Academy of Sciences of the United States of America, 71(2), 469-73 (English) 1974. CODEN: PNASA6. ISSN: 0027-8424.

AB Alk. phosphatase (EC 3.1.3.1) contg. m-fluorotyrosine was prepd. from E. coli grown in the presence of m-fluorotyrosine. The kinetic properties of the m-fluorotyrosine enzyme measured with p-nitrophenylphosphate at pH 8.0 and dinitrophenylphosphate at pH 5.5 are essentially the same as those of normal alk. phosphatase. However, the ability of the m-fluorotyrosine protein to ***refold*** active ***enzyme*** after ***acid*** denaturation, while unchanged at pH 5.8, was markedly decreased at pH 7.6. This result implies that the tyrosines must be in their protonated form for the protein to refold, reassoc., and take on Zn. The 19F NMR spectrum of m-fluorotyrosine alk phosphatase contains resolved resonances corresponding to different chem. environments for each m-fluorotyrosine in the folded protein. This demonstrates that 19F NMR spectroscopy of enzymes specifically labeled with 19F, even with enzymes as large as alk. phosphatase (mol. wt., 86,000), will provide a very valuable probe for conformational changes in proteins.

L30 ANSWER 11 OF 25 CAPLUS COPYRIGHT 2003 ACS

1973:502029 Document No. 79:102029 Transamination reaction catalyzed by kynureninase and control of the enzyme activity. Moriguchi, Mitsuaki; Soda, Kenji (Inst. Chem. Res., Kyoto Univ., Uji, Japan). Biochemistry, 12(16), 2974-9 (English) 1973. CODEN: BICHAW. ISSN: 0006-2960.

AB The inactivation of kynureninase occurred when the reaction was carried out in the absence of added pyridoxal 5'-phosphate. The degree of inactivation increased with the reaction time. The addition of either pyridoxal 5'-phosphate or pyruvate protected the enzyme from inactivation.

Kynureninase was also inactivated by L-alanine, a reaction product, or by L-ornithine. The activity was restored by the addition of pyridoxal 5'-phosphate. Spectrophotometric studies on the inactivation indicate that the addn. of L-alanine or L-ornithine to the holoenzyme leads to loss in the peaks at 337 and 430 m.mu. and appearance of a new peak at 325 m.mu.. Apoenzyme was obtained by dialysis of L-ornithine (or L-alanine)-treated enzyme. The apoenzyme was reactivated by pyridoxamine 5'-phosphate plus pyruvate, or by pyridoxal 5'-phosphate. Thus, the inactivation is due to formation of the bound pyridoxamine 5'-phosphate from the bound pyridoxal 5'-phosphate by transamination with L-alanine or L-ornithine. The product from L-ornithine was .DELTA.1-pyrroline-2-carboxylic acid, the intramolecularly dehydrated form of .alpha.-keto-.DELTA.-aminovaleric acid. Kynureninase catalyzes an overall transamination between L-ornithine and pyruvate. There is close correlation between the amino acids that cause inactivation and those that transaminate, and between the .alpha.-keto ***acids*** that ***reactivate*** the inactivated ***enzyme*** and those that transaminate. The enzyme can act as an .alpha.-aminotransferase of high substrate specificity to regulate the enzyme activity by interconversion of the coenzyme moiety.

L30 ANSWER 12 OF 25 CAPLUS COPYRIGHT 2003 ACS

1971:120488 Document No. 74:120488 Threonine deaminase from Arthrobacter strain 23. Baumgarten, J.; Schlegel, Hans G. (Inst. Mikrobiol., Univ. Goettingen, Goettingen, Fed. Rep. Ger.). Archiv fuer Mikrobiologie, 75(4), 312-26 (German) 1971. CODEN: ARMKA7. ISSN: 0003-9276.

AB Threonine deaminase (EC 4.2.1.16) purified 11-fold from Arthrobacter strain 23 was inhibited by isoleucine, valine, norvaline, norleucine, and to a minor degree by leucine and .alpha.-aminobutyric acid; alanine and .alpha.-aminoisobutyric acid were noninhibitory even at high concns. Substrate saturation curves detd. with isoleucine, valine, norvaline, and norleucine were sigmoid. The Hill coefficient of n = 1.95 indicated that the enzyme contained 2 binding sites for the inhibitors; antagonistic effects between isoleucine and valine or other negative effectors were not obsd. Kinetic data indicated that the same binding sites are used by the negative effectors isoleucine, valine, norvaline and norleucine. Deprivation of effectors induced enzyme inactivation. Incubation with isoleucine, valine, threonine, or combinations of these amino ***acids*** ***reactivated*** the ***enzyme*** .

L30 ANSWER 13 OF 25 CAPLUS COPYRIGHT 2003 ACS

1968:464779 Document No. 69:64779 Hydrazinopropionic acid: a new inhibitor of aminobutyric transaminase and glutamic decarboxylase. Van Gelder, N. M. (Sch. of Med., Tufts Univ., Boston, MA, USA). Journal of Neurochemistry, 15(8), 747-57 (English) 1968. CODEN: JONRA9. ISSN: 0022-3042.

AB This paper deals with the synthesis of 3-pyrazolidone and the biochem. action of hydrazinopropionic acid. The latter compd. is formed upon alk. hydrolysis of 3-pyrazolidone. Hydrazinopropionic acid was found in vitro to be a very potent inhibitor of bacterial aminobutyrate transaminase as well as of aminobutyrate transaminase and glutamate decarboxylase from mouse brain. This inhibition was shown to occur despite the presence of high concns. of pyridoxal phosphate in the incubation media. Injections of 20 mg. hydrazinopropionic acid/kg. into mice resulted in complete inhibition of aminobutyrate transaminase in brain and approx. 20% inactivation of glutamate decarboxylase. This inhibition could not be prevented or antagonized by administration of pyridoxine to the animals. Addn. of pyridoxal phosphate to homogenates of brain from animals treated with hydrazinopropionic ***acid*** also failed to ***reactivate*** the ***enzymes*** . The tentative conclusion reached from these results is that hydrazinopropionic acid has inhibitory action because of its close similarity to .gamma.-aminobutyric acid (GABA) with respect to mol. size, structural configuration, and mol. charge distribution. This can be demonstrated by comparing a Dreiding model of hydrazinopropionic acid with that representing GABA.

L30 ANSWER 14 OF 25 CAPLUS COPYRIGHT 2003 ACS

1968:75313 Document No. 68:75313 Purification and properties of the 3-hydroxyanthranilate oxygenase of Saccharomyces cerevisiae. Heilmann, Hans D.; Lingens, Franz (Univ. Tuebingen, Tuebingen, Fed. Rep. Ger.). Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie, 349(2), 223-30

(German) 1968. CODEN: HSZPAZ. ISSN: 0018-4888.
AB 3-Hydroxyanthranilate oxygenase (EC 1.13.-1.6), which catalyzes the oxidative cleavage of 3-hydroxy-anthranilic acid, was characterized in S. cerevisiae exts. It was neither repressed nor inhibited by quinolinic acid, nicotinic acid, nicotinamide, and NAD; it was not induced by tryptophan. The enzyme required Fe2+ for activity and was optimally active at pH 7. Ascorbic ***acid*** did not ***reactivate*** the ***enzyme*** , whereas addnl. Fe2+ could. Enzyme activity was inhibited by 1,10-phenanthroline and 2,2'-bipyridine. The product of enzymic oxidn. of 3-hydroxyanthranilic acid was 2-amino-3-carboxy-muconic acid 6-semialdehyde. 19 references.

L30 ANSWER 15 OF 25 CAPLUS COPYRIGHT 2003 ACS
1967:450591 Document No. 67:50591 Reversibility of acid inactivation of Bacillus subtilis .alpha.-amylase. Nishida, Akimasa; Fukumoto, Juichiro; Yamamoto, Takehiko (Osaka City Univ., Sumiyoshi, Japan). Agricultural and Biological Chemistry, 31(6), 682-93 (English) 1967. CODEN: ABCHA6. ISSN: 0002-1369.
AB B. subtilis .alpha.-amylase inactivation by acid was reversible. Two different B. subtilis .alpha.-amylases, saccharifying and liquefying types, were used and the reversibility was investigated by dividing into 2 processes of inactivation and reactivation. Both amylases showed the reversibility in a similar degree and in general the ***enzymes*** inactivated by ***acid*** were ***reactivated*** only by adjusting the pH to slightly alk. values followed by incubation under certain conditions. However, the reversibility, esp. the reactivation, was greatly influenced by several chemicals, the effect of certain chemicals such as urea or HCl being different according to the type of the bacterial amylase. Contrary to liquefying amylase, saccharifying amylase was insensitive to metal chelators such as EDTA but, nevertheless, the reatactivion of the amylase was prevented by metal chelators. Also the reactivation of saccharifying amylase was inhibited by SH reagents such as p-chloromercuribenzoate, although the native enzyme was quite insensitive to the chemicals. In the acid inactivation and reactivation process, a reversible change in the uv absorption spectra of the enzymes was observed. 15 references.

L30 ANSWER 16 OF 25 CAPLUS COPYRIGHT 2003 ACS
1965:60346 Document No. 62:60346 Original Reference No. 62:10749e-h,10750a In vitro complementation and the subunit structure of Escherichia coli alkaline phosphatase. Schlesinger, Milton J. (Massachusetts Inst. of Technol., Cambridge). U.S. At. Energy Comm., BNL-869-(C-40), 66-76, discussion 77-9 (English) 1964.
AB Pretreatment of the antigenically related inactive proteins (CRM) with mild acid and reaction at a neutral pH resulted in a 25-fold increase in alk. phosphatase (I) activity over the level of the CRM. Chloromercuribenzoate and iodoacetate had little or no effect on complementation. Trypsin and pronase completely inhibited the formation of active hybrids. The rates of active enzyme formation were proportional to the square of the protein concn. and were independent of Zn++ concn. The metal is suggested to serve as a cofactor in the dimerization reaction and no Zn-free dimer is formed. CRM-forming mutants, U 9, S 19, and U 32, were unable to complement with each other. Gene map distance was not correlated with the amt. of complementation. A complementation map was not related in a simple colinear manner with the gene map. In vivo and in vitro complementation results were similar. The wild-type enzyme required pretreatment at pH 2.3 for complete dissocn. A class of mutants was complemented without preacidification; this class complemented with all other mutants. An equil. with monomer and dimer is thought to exist at neutral pH. A possibility of 2 active sites on the dimer is suggested. The purified enzyme from the wild-type strain of E. coli resisted inactivation until a pH of <3.0; first-order kinetics were involved. At pH 2.3, there was inactivation to 95% of the control enzyme activity. From pH 5.0 to 3.0 the rate was slow and almost unchanged. Under conditions for complementation the ***acid*** -denatured protein can be ***reactivated*** to normal ***enzyme*** . The reaction was temp. dependent, bimolecular, and required Zn++. The mol. wt. of acid-denatured enzyme, at pH 4.0 in EDTA, was 43,000; native enzyme, at pH 7.4, was 86,000. The I monomer was distinguished from the active dimer; it was more labile and selectively "denatured" by trypsin and pronase, heat, and detergents. Diazotized sulfanilic acid and periodate reacted with groups

exposed on the monomer but not on the dimer. Disulfide bonds were found to be of an intra chain type. Polar groups were found "masked" in the native protein. Hydrophobic bonds are thought to be formed during dimerization. The ultraviolet difference spectrum between native and acid-dissocd. enzyme had max. at 233, 279, and 286 m.mu.. When excited at 280 m.mu., native I had an emission fluorescence max. at 343 m.mu.. It was shifted to 360 m.mu. and decreased in intensity when acidified. Reactivation increased the 343 m.mu. emission. The change in emission intensity occurred at the same rate as the appearance of active enzyme. Dimerization of I monomers is suggested to be the direct result of the amino acid sequence in the polypeptide chain.

L30 ANSWER 17 OF 25 CAPLUS COPYRIGHT 2003 ACS
1964:70298 Document No. 60:70298 Original Reference No. 60:12417h,12418a Black light inactivation of transforming deoxyribonucleic acid from Haemophilus influenzae. Cabrera-Juarez, Emiliano (Inst. Politec. Nacl., Mex.). J. Bacteriol., 87(4), 771-8 (Unavailable) 1964.
AB The biol. activity (intrinsic genetic markers or nitrous acidmutable regions) of transforming deoxyribonucleic acid (DNA) from H. influenzae has been inactivated by black light (BL) by 2 mechanisms: (i) photodynamic action (O-dependent) and (ii) BL inactivation (O-independent). The BL inactivation is greater in denatured than in native DNA and it is dependent on the pH. It does not depend on the temp. and the damage produced is stable. The effective wavelength of inactivation is between 330 and 360 m.mu.. The BL inactivation is not ***reactivated*** by photoreactivating ***enzyme*** or nitrous ***acid*** . The BL and ultraviolet inactivations are additive, suggesting that the changes produced by BL and ultraviolet irradiation on transforming DNA are different. T2 phage was also inactivated by BL.

L30 ANSWER 18 OF 25 CAPLUS COPYRIGHT 2003 ACS
1961:76365 Document No. 55:76365 Original Reference No. 55:14532d-e D-Lactic acid dehydrogenase from anaerobic yeast; combination of apoenzyme with zinc and cobalt and comparison of the reconstituted enzymes. Curdel, Andree; Labeyrie, Francoise (Inst. biol. phys.-chim., Paris). Biochem. Biophys. Research Communs., 4, 175-9 (Unavailable) 1961.
AB cf. CA 53, 20269i; 54, 11111c. After complete inactivation by ethylenediaminetetraacetic ***acid*** , the ***enzyme*** ***reactivated*** by Zn++ had a Michaelis const. of 1.7 mM, the same as that of the native enzyme. When reactivated with Co++ the Michaelis const. was 0.15 mM.

L30 ANSWER 19 OF 25 CAPLUS COPYRIGHT 2003 ACS
1957:17566 Document No. 51:17566 Original Reference No. 51:3684f-g Pyridoxal phosphate as coenzyme of diamine oxidase. Davidson, A. N. (Roche Products Ltd., Herts, UK). Biochem. J., 64, 546-8 (Unavailable) 1956.
AB The identity of histaminase and diamine oxidase of pig kidney has been substantiated by the finding that there is no summation of O uptake of cadaverine or histamine when added together to diamine oxidase. The apoenzyme of diamine oxidase could not be sepd. from the coenzyme. Isonicotinic ***acid*** hydrazide inhibited the ***enzyme*** , but pyridoxal phosphate ***reactivated*** the enzyme. Conclusion: Pyridoxal phosphate is probably a coenzyme.

L30 ANSWER 20 OF 25 CAPLUS COPYRIGHT 2003 ACS
1956:24519 Document No. 50:24519 Original Reference No. 50:5061f-i The anticholinesterase activity of arylarsonic and diarylarsinic acids. Freedman, Leon D.; Doak, G. O. (Univ. of North Carolina, Chapel Hill). J. Am. Chem. Soc., 77, 6374-6 (Unavailable) 1955. CODEN: JACSAT. ISSN: 0002-7863.
AB cf. C.A. 48, 3917e. The anticholinesterase activity is reported for a series of aromatic arsonic and arsinic acids. All the arsonic acids showed some activity; there was no correlation between the activities of the corresponding arsonic and phosphonic acids. The order of activity for the halo-substituted arsinic acids is o < m < p; the nitro-substituted arsinic acids showed no activity. The anticholinesterase activity of the arsonic acids differs in several respects from that of the 3 other types of acids studied (loc. cit.). The inhibition of plasma cholinesterase by phosphonic, phosphinic, and arsinic acids can be readily reversed by dialysis; the inactivation by arsonic acids is not changed. The

phosphonic, phosphinic, and arsinic acids react rapidly with plasma cholinesterase; the inhibition by arsonic acids is slow but even when the substrate and arsonic acid are added to the enzyme simultaneously (zero incubation time) there is still appreciable inhibition. The inhibition by arsonic acids resembles that by HgCl2 in some respects; BAL does not ***reactivate*** the ***enzyme*** after inhibition by arsonic ***acids*** or HgCl2. Conclusion: Phosphonic, phosphinic, and arsinic acids inhibit plasma cholinesterase by the same mechanism and arsonic acids inhibit by another mechanism. The following Br-substituted acids were synthesized by a previously described method, compd., percentage yield, and m.p. given: o-BrC6H4AsO3H2.H2O, 29-46, 173-5.degree.; p-BrC6H4AsO3H2.1/2H2O, 35, above 300.degree.; (o-BrC6H4)2AsO2H, 5-12, 275-8.degree.; (p-BrC6H4)2AsO2H, 3, 189-90.degree.; (o-BrC6H4PhAsO2H, 13, 191-8.degree..

L30 ANSWER 21 OF 25 CAPLUS COPYRIGHT 2003 ACS
1955:53793 Document No. 49:53793 Original Reference No. 49:10390c-d Triphosphopyrithiamine and p-chloromercuribenzoate in a study on the prosthetic groups of the pyruvic acid oxidase of animal tissue. Onrust, H.; van der Linden, A. C.; Jansen, B. C. P. (Univ. Amsterdam). Enzymologia, 16, 289-97 (English) 1954.
AB cf. C.A. 47, 6997g; Woolley and Merrifield, Federation Proc. 11, 458(1952). Triphosphopyrithiamine (I) partially inhibits the enzyme in a pigeon-breast muscle prepn.; this inhibition is reversed by yeast. I is not the primary electron acceptor in the pyruvate oxidation. p-Chloromercuribenzoate completely inhibits the ***enzyme*** which is ***reactivated*** by thiomalic ***acid*** and glutathione. The enzyme is SH-dependent, but the SH groups essential for enzyme activity do not originate from diphosphothiamine. The possible role of .alpha.-lipoic acid as a primary electron acceptor is discussed.

L30 ANSWER 22 OF 25 CAPLUS COPYRIGHT 2003 ACS
1939:48124 Document No. 33:48124 Original Reference No. 33:6883c-d Necessity of sulfhydryl groups for the activity of glyceraldehyde dehydrogenase. Trpinac, Pavle Compt. rend. soc. biol., 131, 24-7 (Unavailable) 1939.
AB The oxidized ***enzyme*** is not ***reactivated*** by ascorbic ***acid*** . The enzyme is inactivated by dil. I2 soln. Most of the activity is restored by treatment with cysteine or reduced glutathione. CH2ICO2H irreversibly inactivates the enzyme. Maleic acid destroys most of the activity; fumaric acid has no effect.

L30 ANSWER 23 OF 25 CAPLUS COPYRIGHT 2003 ACS
1937:19228 Document No. 31:19228 Original Reference No. 31:2698c-d Vitamin C (l-ascorbic acid) in tea. Golyanitskii, I. A.; Bryushkova, K. A. Compt. rend. acad. sci. U. R. S. S. [N. S.], 4, 381-4 (German) 1936.
AB The vitamin C content of Russian tea and of fresh green tea leaves was detd. by a modification of Karrer's method (cf. C. A. 28, 1389.3). Thirty-one patients with scorbutic myositis, arthritis and hemorrhagia were given enough tea in their diets to furnish 50-60 mg. vitamin C daily (10 to 15 g. tea), and good results obtained in all cases within 6 to 14 days. The tea leaves vary in vitamin C content with the position of the leaf on the plant and with its water content; much is lost in com. prepn. Fermented tea contains more than dried leaves; 12.degree. is the optimum temp. for fermentation. Inactivated l-ascorbic ***acid*** can be ***reactivated*** by ***enzymes*** .

L30 ANSWER 24 OF 25 CAPLUS COPYRIGHT 2003 ACS
1908:13590 Document No. 2:13590 Original Reference No. 2:2958e-i,2959a-b The Hydrolysis of Amygdalin by Emulsin. I. Auld, S. J. M. J. Chem. Soc., 93, 1251-75 (Unavailable) 1908.
AB As the enzymes of Phaseolus lunatus, flax, and cassava all act in the same manner, it sufficed to use one of them only in the experiments. Java beans are thoroughly dried in the air, ground, and soaked with 3 volumes of water and 1% toluene. After several hrs., the liquid is filtered and allowed to stand in a vacuum desiccator over stick KOH until all the HCN has been absorbed. The liquid is then poured into excess of alcohol, the precipitated enzyme quickly filtered, washed with alcohol, dried in a vacuum and powdered. If the powder does not completely dissolve in water, the process of solution and precipitation must be repeated. The powder remains active for months. By carefully heating an enzyme solution and

observing the relative rates of decomposition of amygdalin and phaseolunatin, it is observed that the activity toward the latter is destroyed before that which causes the decomposition of the .beta.-glucosides. The author concludes that there are present in the "phaseolunatase" two enzymes, which he designates the .alpha.- and .beta.-enzymes, corresponding with their activity toward .alpha.- and .beta.-glucosides. By measuring only the initial velocities induced by varying quantities of emulsin in separate solutions, and thus avoiding any complications due to secondary action of the decomposition products, it was found that with small concentrations of the enzyme the velocity of hydrolysis is proportional to the concentration of emulsin. As the concentration is increased this relationship ceases, and eventually a further increase in the quantity of emulsin present produces no corresponding increase in velocity. The velocity of reaction is independent of the concentration of amygdalin when the latter is present in large excess. Thus a constant quantity, and not a constant fraction, of amygdalin is hydrolyzed in unit time. Very large quantities of amygdalin begin to produce a retarding effect on the action. The reaction is retarded also by addition of the products of the reaction; benzaldehyde and HCN have greater retarding effect than dextrose. HCN probably retards this action as a weak acid, inasmuch as both acids and bases inhibit the hydrolysis. HCN in extremely small quantities has a slight accelerating effect, but it is not comparable with the effect of acid solutions on peptic proteolysis. Emulsin seems therefore to possess its optimum activity in neutral solution. Mineral acids or alkalis do not entirely destroy the activity; neutralization by alkalis or ***acids*** ***reactivates*** the ***enzyme*** to a large extent. The hydrolysis is more complete than is generally assumed; in only 22 hrs. 93.3% decomposition was recorded. Attempts to test the synthetic action of emulsin were unsuccessful. The temperature coefficient kt+10/kt only twice rose above 2.0 from 15-60.degree.. The method for the measurement of the hydrolysis consisted in estimating the HCN by titration with a stand and I solution. Figures of the apparatus are given.

L30 ANSWER 25 OF 25 CAPLUS COPYRIGHT 2003 ACS

1908:13589 Document No. 2:13589 Original Reference No. 2:2958e-i,2959a-b The Hydrolysis of Amygdalin by Emulsin. I. Auld, S. J. M. (Chem. Dept., East London Coll.). Proc. Chem. Soc., 24, 97 (Unavailable) 1908.

AB As the enzymes of Phaseolus lunatus, flax, and cassava all act in the same manner, it sufficed to use one of them only in the experiments. Java beans are thoroughly dried in the air, ground, and soaked with 3 volumes of water and 1% toluene. After several hrs., the liquid is filtered and allowed to stand in a vacuum desiccator over stick KOH until all the HCN has been absorbed. The liquid is then poured into excess of alcohol, the precipitated enzyme quickly filtered, washed with alcohol, dried in a vacuum and powdered. If the powder does not completely dissolve in water, the process of solution and precipitation must be repeated. The powder remains active for months. By carefully heating an enzyme solution and observing the relative rates of decomposition of amygdalin and phaseolunatin, it is observed that the activity toward the latter is destroyed before that which causes the decomposition of the .beta.-glucosides. The author concludes that there are present in the "phaseolunatase" two enzymes, which he designates the .alpha.- and .beta.-enzymes, corresponding with their activity toward .alpha.- and .beta.-glucosides. By measuring only the initial velocities induced by varying quantities of emulsin in separate solutions, and thus avoiding any complications due to secondary action of the decomposition products, it was found that with small concentrations of the enzyme the velocity of hydrolysis is proportional to the concentration of emulsin. As the concentration is increased this relationship ceases, and eventually a further increase in the quantity of emulsin present produces no corresponding increase in velocity. The velocity of reaction is independent of the concentration of amygdalin when the latter is present in large excess. Thus a constant quantity, and not a constant fraction, of amygdalin is hydrolyzed in unit time. Very large quantities of amygdalin begin to produce a retarding effect on the action. The reaction is retarded also by addition of the products of the reaction; benzaldehyde and HCN have greater retarding effect than dextrose. HCN probably retards this action as a weak acid, inasmuch as both acids and bases inhibit the hydrolysis. HCN in extremely small quantities has a slight accelerating effect, but it is not comparable with the effect of acid solutions on

peptic proteolysis. Emulsin seems therefore to possess its optimum activity in neutral solution. Mineral acids or alkalis do not entirely destroy the activity; neutralization by alkalis or ***acids*** ***reactivates*** the ***enzyme*** to a large extent. The hydrolysis is more complete than is generally assumed; in only 22 hrs. 93.3% decomposition was recorded. Attempts to test the synthetic action of emulsin were unsuccessful. The temperature coefficient kt+10/kt only twice rose above 2.0 from 15-60.degree.. The method for the measurement of the hydrolysis consisted in estimating the HCN by titration with a stand and I solution. Figures of the apparatus are given.

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=> E YOKOYAMA K/AU
=> S E3,E76-E80
           266 "YOKOYAMA K"/AU
             7 "YOKOYAMA KEI"/AU
             9 "YOKOYAMA KEI ICHI"/AU
             1 "YOKOYAMA KEICHI"/AU
             1 "YOKOYAMA KEIICH"/AU
           195 "YOKOYAMA KEIICHI"/AU
L31        479 ("YOKOYAMA K"/AU OR "YOKOYAMA KEI"/AU OR "YOKOYAMA KEI ICHI"/AU
               OR "YOKOYAMA KEICHI"/AU OR "YOKOYAMA KEIICH"/AU OR "YOKOYAMA
               KEIICHI"/AU)

=> E ONO K/AU
=> S E3
L32        469 "ONO K"/AU

=> E ONO KU/AU
=> S E12
L33         20 "ONO KUNIO"/AU

=> E EJIMA D/AU
=> S E3,E4
             1 "EJIMA D"/AU
            22 "EJIMA DAISUKE"/AU
L34         23 ("EJIMA D"/AU OR "EJIMA DAISUKE"/AU)

=> S L31,L32,L33,L34
L35        983 (L31 OR L32 OR L33 OR L34)

=> S L35 AND L4;S L35 AND (L10,L11,L12)
L36         17 L35 AND L4


L37          8 L35 AND ((L10 OR L11 OR L12))

=> S L36,L37
L38         22 (L36 OR L37)

=> S L38 NOT (L16,L30)
L39         19 L38 NOT ((L16 OR L30))

=> D 1-19 CBIB ABS

L39  ANSWER 1 OF 19  CAPLUS  COPYRIGHT 2003 ACS
2003:48266  Secretion of active-form Streptoverticillium mobaraense
       ***transglutaminase***  by Corynebacterium glutamicum: Processing of the
     pro- ***transglutaminase***  by a cosecreted subtilisin-like protease
     from Streptomyces albogriseolus. Kikuchi, Yoshimi; Date, Masayo;
       ***Yokoyama, Kei-ichi***  ; Umezawa, Yukiko; Matsui, Hiroshi (Institute of
     Life Sciences, Ajinomoto Co., Inc., Kawasaki, 210-8681, Japan). Applied
     and Environmental Microbiology, 69(1), 358-366 (English) 2003. CODEN:
     AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.
AB   The ***transglutaminase***  secreted by Streptoverticillium mobaraense
     is a useful enzyme in the food industry. A fragment of
       ***transglutaminase***  was secreted by Corynebacterium glutamicum when
     it was coupled on a plasmid to the promoter and signal peptide of a cell
     surface protein from C. glutamicum. We analyzed the signal peptide and
     the pro-domain of the ***transglutaminase***  gene and found that the
     signal peptide consists of 31 amino acid residues and the pro-domain
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consists of 45 residues. When the pro-domain of the ***transglutaminase*** was used, the pro- ***transglutaminase*** was secreted efficiently by C. glutamicum but had no enzymic activity. However, when the plasmid carrying the S. mobaraense ***transglutaminase*** also encoded SAM-P45, a subtilisin-like serine protease derived from Streptomyces albogriseolus, the peptide bond to the C side of 41-Ser of the pro- ***transglutaminase*** was hydrolyzed, and the pro- ***transglutaminase*** was converted to an active form. Our findings suggest that C. glutamicum has potential as a host for industrial-scale protein prodn.

L39 ANSWER 2 OF 19 CAPLUS COPYRIGHT 2003 ACS
2002:862753 Crystal Structure of Microbial ***Transglutaminase*** from Streptoverticillium mobaraense. Kashiwagi, Tatsuki; ***Yokoyama,*** *** Kei-ichi*** ; Ishikawa, Kohki; ***Ono, Kunio*** ; ***Ejima,*** *** Daisuke*** ; Matsui, Hiroshi; Suzuki, Ei-ichiro (Ajinomoto Company Inc., Central Research Laboratories, Kawasaki-ku, Kawasaki, Kanagawa, 210-8681, Japan). Journal of Biological Chemistry, 277(46), 44252-44260 (English) 2002. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.
AB The crystal structure of a microbial ***transglutaminase*** from Streptoverticillium mobaraense has been detd. at 2.4 .ANG. resoln. The protein folds into a plate-like shape, and has one deep cleft at the edge of the mol. Its overall structure is completely different from that of the factor XIII-like ***transglutaminase*** , which possesses a cysteine protease-like catalytic triad. The catalytic residue, Cys64, exists at the bottom of the cleft. Asp255 resides at the position nearest to Cys64 and is also adjacent to His274. Interestingly, Cys64, Asp255, and His274 superimpose well on the catalytic triad "Cys-His-Asp" of the factor XIII-like ***transglutaminase*** , in this order. The secondary structure frameworks around these residues are also similar to each other. These results imply that both ***transglutaminases*** are related by convergent evolution; however, the microbial ***transglutaminase*** has developed a novel catalytic mechanism specialized for the crosslinking reaction. The structure accounts well for the catalytic mechanism, in which Asp255 is considered to be enzymically essential, as well as for the causes of the higher reaction rate, the broader substrate specificity, and the lower deamidation activity of this enzyme.

L39 ANSWER 3 OF 19 CAPLUS COPYRIGHT 2003 ACS
2002:639964 Comparison of enzymic properties of microbial ***transglutaminase*** from Streptomyces sp. Umezawa, Yukiko; Ohtsuka, Tomoko; ***Yokoyama, Keiichi*** ; Nio, Noriki (Food Research and Development Laboratories, Ajinomoto Co., Inc., Kanagawa, 210-8681, Japan). Food Science and Technology Research, 8(2), 113-118 (English) 2002. CODEN: FSTRFS. ISSN: 1344-6606. Publisher: Japanese Society for Food Science and Technology.
AB Microbial ***transglutaminase*** (TGase) from S. libani was purified from its culture broth and its enzymic properties were compared with those of TGase from Streptoverticillium mobaraense. TGase was purified by ion-exchange chromatog. and size-exclusion chromatog. The specific activity of the main component was 10.7 units/mg protein, lower than that of Streptoverticillium mobaraense (25 units/mg). Several differences in enzymic properties were found between the 2 enzymes. The optimum temp., stability, and gelation activity of TGase from S. libani were lower than those of TGase from S. mobaraense, whereas the deamidation activity was higher. In addn., the existence of some TGases with different pI values were suggested.

L39 ANSWER 4 OF 19 CAPLUS COPYRIGHT 2003 ACS
2002:302586 Document No. 137:59460 Enhancement of ***transglutaminase*** activity by NMR identification of its flexible residues affecting the active site. Shimba, Nobuhisa; Shinohara, Mina; ***Yokoyama,*** *** Kei-ichi*** ; Kashiwagi, Tatsuki; Ishikawa, Kohki; ***Ejima, Daisuke*** ; Suzuki, Ei-ichiro (Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki-ku, Kawasaki-shi, 210-8681, Japan). FEBS Letters, 517(1-3), 175-179 (English) 2002. CODEN: FEBLAL. ISSN: 0014-5793. Publisher: Elsevier Science B.V..
AB Incorporation of inter- or intramol. covalent cross-links into food proteins with microbial ***transglutaminase*** (MTG) improves the phys. and textural properties of many food proteins, such as tofu, boiled

fish paste, and sausage. By using NMR, we have shown that the residues exhibiting relatively high flexibility in MTG are localized in the N-terminal region; however, the N-terminal region influences the microenvironment of the active site. These results suggest that the N-terminal region is not of primary importance for the global fold, but influences the substrate binding. Therefore, in order to increase the ***transglutaminase*** activity, the N-terminal residues were chosen as candidates for site-directed replacement and deletion. We obtained several mutants with higher activity, del1-2, del1-3, and S2R. We propose a strategy for enzyme engineering targeted toward flexible regions involved in the enzymic activity. In addn., we also briefly describe how the no. of glutamine residues in a substrate protein can be increased by mixing more than two kinds of TGases with different substrate specificities.

L39 ANSWER 5 OF 19 CAPLUS COPYRIGHT 2003 ACS

2002:142890 Document No. 136:196189 Method of modifying microbial ***transglutaminase*** substrate specificity by x-ray crystal structure-based designing and mutagenesis. Kashiwagi, Tatsuki; Shimba, Nobuhisa; Ishikawa, Kohki; Suzuki, Eiichiro; ***Yokoyama, Keiichi*** ; Hirayama, Kazuo (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2002014518 A1 20020221, 126 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2001-JP7038 20010815. PRIORITY: JP 2000-247664 20000817; JP 2000-396695 20001227.

AB A method of designing and constructing a variant ***transglutaminase*** of Streptoverticillium mobaraense origin based on the three dimensional stereo-structure, for modifying substrate specificity and improving reactivity, is disclosed. The variant ***transglutaminase*** is designed and constructed by estg. the substrate-binding site of ***transglutaminase*** based on the stereo-structure obtained by anal. of the x-ray cryst. structure, and substituting, inserting or deleting the amino acid residue located at the substrate-binding site of the ***transglutaminase*** . Acidic amino acids, in particular, are deleted. Recombinant expression of the modified MTG. ***Transglutaminase*** crystals of the monoclinic system with P21 space group, are claimed. Expression of recombinant ***transglutaminase*** in E. coli and detn. of x-ray cryst. structure, are described. N-terminal Asp deletion mutant, mutants with Ser2 substituted with Tyr, Arg, or Asp, N-terminal 2 or 3 residues deletion mutants, mutants with residues 241-252 or 278-287 substituted with 3 Gly residues, were constructed.

L39 ANSWER 6 OF 19 CAPLUS COPYRIGHT 2003 ACS

2002:114386 Document No. 136:199144 NMR-Based Screening Method for ***Transglutaminases*** : Rapid Analysis of Their Substrate Specificities and Reaction Rates. Shimba, Nobuhisa; ***Yokoyama, Kei-ichi*** ; Suzuki, Ei-ichiro (Central Research Laboratories, Ajinomoto Company, Kawasaki-ku, Kawasaki-shi, 210-8681, Japan). Journal of Agricultural and Food Chemistry, 50(6), 1330-1334 (English) 2002. CODEN: JAFCAU. ISSN: 0021-8561. Publisher: American Chemical Society.

AB Incorporation of inter- or intramol. covalent cross-links into food proteins with microbial ***transglutaminase*** (MTG) improves the phys. and textural properties of many food proteins such as tofu, boiled fish paste, and sausage. Other ***transglutaminases*** (TGases) are expected to be used in the same way, and also to extend the scope of industrial applications to materials, drugs, and so on. The TGases have great diversity, not only in amino acid sequence and size, but also in their substrate specificities and catalytic activities, and therefore, it is quite difficult to est. their reactivity. We have developed an NMR-based method using the enzymic labeling technique (ELT) for simultaneous anal. of the substrate specificities and reaction rates of TGases. It is quite useful for comparing the existing TGases and for screening new TGases or TGases variants. This method has shown that MTG is superior for industrial use because of its lower substrate specificity compared with those of guinea pig liver ***transglutaminase*** (GTG)

and red sea bream liver ***transglutaminase*** (FTG). We have also found that an MTG variant lacking an N-terminal aspartic acid residue has higher activity than that of the native enzyme.

L39 ANSWER 7 OF 19 CAPLUS COPYRIGHT 2003 ACS
2002:73594 Document No. 137:17355 Enzymatic labeling of arbitrary proteins. Shimba, Nobuhisa; Yamada, Naoyuki; ***Yokoyama, Kei-ichi*** ; Suzuki, Ei-ichiro (Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki-ku, Kawasaki-shi, 210-8681, Japan). Analytical Biochemistry, 301(1), 123-127 (English) 2002. CODEN: ANBCA2. ISSN: 0003-2697. Publisher: Academic Press.
AB This paper describes an enzymic labeling technique (ELT), using ***transglutaminases*** . On the basis of the ELT, isotopic nuclei are easily incorporated into the .gamma.-carboxyamide groups of glutamine residues in arbitrary proteins, without changing their chem. structures. We have also shown that, by using ELT, protein aggregation was easily checked for NMR studies and that it can be applicable for the screening of weakly bound ligands for proteins. Owing to the simple prepn. of the isotope-labeled proteins, ELT should be useful for speeding up various structural and functional analyses of arbitrary proteins. (c) 2002 Academic Press.

L39 ANSWER 8 OF 19 CAPLUS COPYRIGHT 2003 ACS
2001:850829 Document No. 135:368957 Method for isotope labeling of protein with enzyme. Shimba, Nobuhisa; Suzuki, Eiichiro; ***Yokoyama,*** *** Keiichi*** (Ajinomoto Co., Inc., Japan). Eur. Pat. Appl. EP 1156330 A2 20011121, 16 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 2001-111690 20010514. PRIORITY: JP 2000-141152 20000515.
AB The present invention provides a method for isotopically labeling a functional group possessed by an amino acid residue of a protein. The present invention also provides a protein whose functional group in an amino acid residue is isotopically labeled. A functional group in an amino acid residue of a protein is substituted with an isotope-labeling group derived from an isotope-labeling compd. by making use of the action of an enzyme. In particular, the carboxyamide nitrogen atom in a glutamine residue of a protein is replaced with an isotopically labeled atom by acting a ***transglutaminase*** on the glutamine residue.

L39 ANSWER 9 OF 19 CAPLUS COPYRIGHT 2003 ACS
2001:842069 Document No. 135:354451 Modification of proteins for structural analysis. Shinba, Nobuhisa; Suzuki, Eiichiro; ***Yokoyama, Keiichi*** (Ajinomoto Co., Inc., Japan). Jpn. Kokai Tokkyo Koho JP 2001321192 A2 20011120, 11 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2000-141151 20000515.
AB Proteins are modified with ***transglutaminase*** in the presence of primary amines or glutamine residue-contg. peptides. The modified proteins are useful for structural anal. with X-ray and NMR. Modification of bovine serum albumin (BSA) with glycine was shown. The modified BSA has better soly. and stability under higher temp.

L39 ANSWER 10 OF 19 CAPLUS COPYRIGHT 2003 ACS
2001:658741 Document No. 136:353831 Application of combined reagent solution to the oxidative ***refolding*** of recombinant human interleukin 6. Harada, Takushi; Kurimoto, Eiji; Moriyama, Yuji; ***Ejima, Daisuke*** ; Sakai, Tomoya; Nohara, Daisuke; Kato, Koichi (Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, 467-8603, Japan). Chemical & Pharmaceutical Bulletin, 49(9), 1128-1131 (English) 2001. CODEN: CPBTAL. ISSN: 0009-2363. Publisher: Pharmaceutical Society of Japan.
AB Human interleukin 6 (hIL-6), which is a cytokine involved in diverse biol. activities, consists of a 4-helix bundle with 2 disulfide bonds. For the clin. use of hIL-6 in cancer therapy, designing of com.-scale prodn. systems of recombinant hIL-6 (rhIL-6) expressed by E. coli has been attempted. Since rhIL-6 has been produced as inclusion bodies in the expression systems reported to date, establishment of a strategy to achieve a high yield of ***refolding*** of this recombinant protein is quite desirable. It has been reported that oxidn. of rhIL-6 under a completely denaturing conditions suppresses aggregation during the ***refolding*** process. In this protocol, however, small but

significant amts. of unidentified byproducts unavoidably arose, which might be problematic in the therapeutic use of rhIL-6. Here, detailed characterization of the individual byproducts has been performed on inspection of peptide maps, and the byproducts found to originate from improperly formed disulfide bonds, most of which are disulfide-linked dimers. In order to minimize these byproducts, combined solns. of urea and LiCl were used for oxidative ***refolding*** of rhIL-6. It was demonstrated that combined use of 1-2 M urea and 1-3 M LiCl effectively suppresses the formation of the byproducts as well as aggregates. The authors propose that the use of the combined reagents can be an alternative method for ***refolding*** of rhIL-6 for clin. purposes.

L39 ANSWER 11 OF 19 CAPLUS COPYRIGHT 2003 ACS

2001:318653 Document No. 135:73296 Crystal structure of red sea bream ***transglutaminase*** . Noguchi, Kazuyoshi; Ishikawa, Kohki; ***Yokoyama, Kei-Ichi*** ; Ohtsuka, Tomoko; Nio, Noriki; Suzuki, Ei-Ichiro (Central Research Laboratories, Ajinomoto Company Inc., Kanagawa, 210-8681, Japan). Journal of Biological Chemistry, 276(15), 12055-12059 (English) 2001. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB The crystal structure of the tissue-type ***transglutaminase*** from red sea bream liver (fish-derived ***transglutaminase*** , FTG) has been detd. at 2.5-.ANG. resoln. using the mol. replacement method, based on the crystal structure of human blood coagulation factor XIII, which is a ***transglutaminase*** zymogen. The model contains 666 residues of a total of 695 residues, 382 water mols., and 1 sulfate ion. FTG consists of four domains, and its overall and active site structures are similar to those of human factor XIII. However, significant structural differences are obsd. in both the acyl donor and acyl acceptor binding sites, which account for the difference in substrate preferences. The active site of the enzyme is inaccessible to the solvent, because the catalytic Cys 272 hydrogen-bonds to Tyr 515, which is thought to be displaced upon acyl donor binding to FTG. It is postulated that the binding of an inappropriate substrate to FTG would lead to inactivation of the enzyme because of the formation of a new disulfide bridge between Cys 272 and the adjacent Cys 333 immediately after the displacement of Tyr 515. Considering the mutational studies previously reported on the tissue-type ***transglutaminases*** , the authors propose that Cys 333 and Tyr 515 are important in strictly controlling the enzymic activity of FTG.

L39 ANSWER 12 OF 19 CAPLUS COPYRIGHT 2003 ACS

2001:247521 Document No. 134:291099 Recombinant expression and extracellular secretion of exogenous proteins in coryneform bacteria by protease cleavage of proprotein-signal peptide fusion construct. Kikuchi, Yoshimi; Date, Masayo; Umezawa, Yukiko; ***Yokoyama, Keiichi*** ; Matsui, Hiroshi (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 2001023591 A1 20010405, 151 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2000-JP6780 20000929. PRIORITY: JP 1999-280098 19990930; JP 2000-194043 20000628.

AB A process for the prodn. of a exogenous secretory protein by using a coryneform bacterium is disclosed. The method comprises making a coryneform bacterium to produce an industrially useful exogenous protein (in particular, ***transglutaminase***) and efficiently secreting the product outside the cells (i.e., secretion). A target exogenous protein is produced by using an expression construct wherein the target exogenous protein gene sequence contg. the pro-structure part is ligated to the downstream of a sequence encoding the signal peptide originating in a coryneform bacterium, transferring this expression type gene construct into the coryneform bacterium, culturing the thus transformed coryneform bacterium, and treating the extracellularly released protein with a protease, etc. to cleave and eliminate the pro-part. Use of the signal peptide of S-layer protein (S-protein) such as Corynebacterium ammoniagenes slpA or Corynebacterium glutamicum PS1 and PS2, with a Streptomyces albogriseolus serine protease SAM-P45 and Streptomyces

mobaraense proline-specific peptidase svPEP, for the prodn. of Streptoverticillium mobaraense or Streptoverticillium cinnamoneum pro- ***transglutaminase*** , is described. Streptomyces mobaraense proline-specific peptidase svPEP, active toward Ala-Ala-Pro-pNA, Ala-Phe-Pro-pNA, and Phe-Arg-Ala-Pro-pNA, and inhibited by phenylmethyl sulfonyl fluoride (PMSF) or aminoethyl benzene sulfonyl fluoride hydrochloride, is specifically used.

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1999:764069 Document No. 132:10493 Process for purification of human activin. ***Ono, Kunio*** ; Tsuchiya, Shigekatsu; ***Ejima,*** *** Daisuke*** ; Eto, Yuzuru (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 9961474 A1 19991202, 30 pp. DESIGNATED STATES: W: US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1999-JP2680 19990520. PRIORITY: JP 1998-159943 19980525.
AB Described is a process for purifying human activin by using cation exchange chromatog. involving the chaotropic ion concn. gradient elution method. By using this process, highly pure human activin A suitable for medicinal utilization can be produced on an industrial scale. Purifn. of human activin A from a culture of transgenic Escherichia coli with a process including ***refolding*** , membrane-facilitated condensation, and chromatog. on Sephadex G25-M was also demonstrated.

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1999:42538 Document No. 130:109259 Manufacture of a ***transglutaminase*** of Streptoverticillium by expression of a synthetic gene in Escherichia coli. ***Yokoyama, Keiichi*** ; Nakamura, Nami; Miwa, Tetsuya; Seguro, Katsuya (Ajinomoto Co., Inc., Japan). Eur. Pat. Appl. EP 889133 A2 19990107, 56 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 1998-112315 19980702. PRIORITY: JP 1997-180010 19970704.
AB A method for efficient manuf. of a bacterial ***transglutaminase*** in Escherichia coli is described. The method uses a synthetic gene for the enzyme with minor alterations at the N-terminus (deletion of the aspartic acid at position 2) that allow efficient processing by the host methionine aminopeptidase. The gene is expressed from the strong promoter of the trp operon in a multicopy plasmid. A gene in which several of the arginine codons had been changed to those found in highly expressed Escherichia coli genes was constructed and placed under control of the trp promoter. The protein accumulated as inclusion bodies with yields of .gtoreq.300 mg/L of protein. The protein was not efficiently processed to remove the N-terminal methionine or N-formylmethionine. When the aspartic acid residue was deleted, processing to leave an N-terminal serine was 90% complete. The enzyme retained its normal specific activity.

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1999:29972 Document No. 130:179454 High yield ***refolding*** and purification process for recombinant human interleukin-6 expressed in Escherichia coli. ***Ejima, Daisuke*** ; Watanabe, Mayumi; Sato, Yutaka; Date, Masayo; Yamada, Naoyuki; Takahara, Yoshiyuki (Central Research Laboratories, Ajinomoto Company, Inc., Kawasaki-ku, Kawasaki, 210-8681, Japan). Biotechnology and Bioengineering, 62(3), 301-310 (English) 1999. CODEN: BIBIAU. ISSN: 0006-3592. Publisher: John Wiley & Sons, Inc..
AB Recombinant human interleukin-6 (hIL-6), a pleiotropic cytokine contg. two intramol. disulfide bonds, was expressed in Escherichia coli as an insol. inclusion body, before being ***refolded*** and purified in high yield providing sufficient qualities for clin. use. Quant. reconstitution of the native disulfide bonds of hIL-6 from the fully denatured E. coli exts. could be performed by glutathione-assisted oxidn. in a completely denaturating condition (6M guanidinium chloride) at protein concns. higher than 1 mg/mL, preventing aggregation of reduced hIL-6. Oxidn. in 6M guanidinium chloride (GdnHCl) required remarkably low concns. of glutathione (reduced form, 0.01 mM; oxidized form, 0.002 mM) to be added to the solubilized hIL-6 before the incubation at pH 8.5, and 22.degree.C for 16 h. After completion of ***refolding*** by rapid transfer of oxidized hIL-6 into acetate buffer by gel filtration chromatog., residual contaminants including endotoxin and E. coli proteins were efficiently removed by successive steps of chromatog. The amt. of dimeric hIL-6s,

thought to be purifn. artifacts, was decreased by optimizing the salt concns. of the loading materials in the ion-exchange chromatog., and gradually removing org. solvents from the collected fractions of the preparative reverse-phase HPLC. These ***refolding*** and purifn. processes, which give an overall yield as high as 17%, seem to be appropriate for the com. scale prodn. of hIL-6 for therapeutic use.

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1998:454111 Document No. 129:186118 Overproduction of DnaJ in Escherichia coli improves in vivo solubility of the recombinant fish-derived ***transglutaminase*** . ***Yokoyama, Kei-Ichi*** ; Kikuchi, Yoshimi; Yasueda, Hisashi (Food Research and Development Laboratories, Ajinomoto Co. Inc., Kanagawa, 210-0801, Japan). Bioscience, Biotechnology, and Biochemistry, 62(6), 1205-1210 (English) 1998. CODEN: BBBIEJ. ISSN: 0916-8451. Publisher: Japan Society for Bioscience, Biotechnology, and Agrochemistry.
AB The overexpression of red sea bream (Pagrus major) ***transglutaminase*** (TGase, E.C. 2.3.2.13) in Escherichia coli mostly leads to the accumulation of biol. inactive enzyme. Although the soly. of the gene products could be improved by cultivation at a lower temp. (26-28.degree.), most of the synthesized TGase was still in the form of insol. aggregates. The effects of overprodn. of mol. chaperones on the intracellular soly. of newly produced recombinant TGase were examd. The over-expression of dnaK or groES/EL did not improve soly. However, DnaJ greatly increased the soly. of the recombinant TGase, resulting in active enzyme in the presence of calcium ions. Co-expression of dnaK along with dnaJ further increased the content of sol. TGase. Under our exptl. conditions, supplementation with both DnaJ and DnaK elevated the TGase activity in the producer cells by roughly 4-fold, compared with the control strain cultured at 30.degree.. Thus, the authors found that DnaJ is important in controlling the soly. of protein overproduced in E. coli.

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1997:506707 Document No. 127:108058 Method for ***refolding*** human activin A. ***Ejima, Daisuke*** ; ***Ono, Kunio*** ; Sasaki, Michiro; Eto, Yuzuru; Tsuchiya, Shigekatsu (Ajinomoto Co., Inc., Japan; Ejima, Daisuke; Ono, Kunio; Sasaki, Michiro; Eto, Yuzuru; Tsuchiya, Shigekatsu). PCT Int. Appl. WO 9723638 A1 19970703, 28 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1996-JP3700 19961219. PRIORITY: JP 1995-333070 19951221.
AB A method for industrially producing human activin A which comprises ***refolding*** a modified human activin A produced by a microorganism into natural-form human activin A having a biol. activity. This method comprises the steps of: (a) solubilizing the modified human activin A with a modifier and then protecting the thiol groups with glutathione and/or sodium sulfite; and (b) dialyzing the protected modified human activin A with a ***refolding*** buffer and then adding a thiol compd. thereto to conduct a disulfide bond interchange reaction.

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1996:751867 Document No. 126:15528 Efficient production of soluble ***transglutaminase*** through co-transformation of Escherichia coli with heat shock protein DnaJ. ***Yokoyama, Keiichi*** ; Kikuchi, Yoshimi; Yasueda, Hisashi (Ajinomoto Co., Inc., Japan). Eur. Pat. Appl. EP 743365 A2 19961120, 13 pp. DESIGNATED STATES: R: DE, FR, GB, IT. (English). CODEN: EPXXDW. APPLICATION: EP 1996-107929 19960517. PRIORITY: JP 1995-118067 19950517.
AB The present invention relates to a process for producing a ***transglutaminase*** (I), which comprises incubating Escherichia coli expressing genes encoding a heat shock protein (DnaJ) and a ***transglutaminase*** . Red sea bream I recombinant DNA using E. coli as a host forms inclusion bodies and inactive enzyme. Co-transformation with heat-shock chaperones such as GroEL, GroES, and DnaK was not sufficient to suppress the insolubilization, and moreover, appeared to inhibit the growth of the host cells. However, DnaJ, is effective for solubilizing and accumulating the desired I in cells; the effect is stabilized when DnaJ and DnaK coexist. The I is produced in large quantities, at low cost, and has the appropriate stereostructure to render the ***transglutaminase*** biol. active. The I so produced is useful in the food industry.

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1992:590277 Document No. 117:190277 Purification of human B cell differentiation factor. ***Ejima, Daisuke*** ; Sato, Yutaka; Watanabe, Mayumi; Date, Masayo; Takahara, Yoshiyuki (Ajinomoto Co., Inc., Japan). PCT Int. Appl. WO 9214832 A1 19920903, 59 pp. DESIGNATED STATES: W: JP, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LU, MC, NL, SE. (Japanese). CODEN: PIXXD2. APPLICATION: WO 1992-JP204 19920225. PRIORITY: JP 1991-115689 19910226; JP 1992-29525 19920217.

AB A process for purifying human B cell differentiation factor (BCDF) from the cultured microorganism expressing the BCDF gene is described. The process comprises solubilizing the product with guanidine HCl, oxidn., ***refolding*** , followed by gel filtration chromatog.in the presence of 4-7 M guanidine HCl. A method for removing org. solvents during purifn. is also described. The resulting BCDF exhibits intramol. disulfide linkage and stereochem. of the natural BCDF. A study showed the content of endotoxin in the BCDF purified from Escherichia coli was <0.01 EU/mg BCDF and the yield was 15%.